3059-Pos Board B164
Cardiac Myofibroblast-Myocyte Gap Junction Coupling Promotes After Depolarizations
Thao P. Nguyen, Yuanfang Xie, Alan Garfinkel, Zhilin Qu, James N. Weiss.
Tissue fibrosis, as seen in diseased and aged hearts, promotes ventricular arrhythmias. We hypothesize that de novo gap junction coupling between myofibroblasts, which proliferate following cardiac stress injury, with neighboring ventricular myocytes may facilitate arrhythmia triggers such as early afterdepolarizations (EADs). A novel hybrid computational and biological approach was employed: virtual fibroblasts with programmable properties embedded in the dynamic clamp were coupled to a real patch-clamped rabbit ventricular myocyte exposed to oxidative (0.1 mM H_2O_2) or ionic (2.7 mM K^+_o) stressors. A virtual gap junction current of programmable conductance was added in real time to myocyte currents and their combined effects on myocyte action potentials and EAD genesis were evaluated. Exposure of myocytes to basal conditions failed to induce EADs, whereas exposure to stress led to 'bradycardia-dependent' EADs that occurred only during slow, but not during fast, pacing. However, when stressed myocytes were then coupled to a virtual fibroblast, EADs emerged independently of pacing rates. Fibroblast coupling alone to unstressed myocytes failed to induce EADs at any pacing rate. The virtual gap junction current has 2 components, but the earlier transient outward component was most critical for EAD generation; EADs disappeared when the virtual fibroblast was uncoupled from the myocyte during the initial 100 ms of the AP, but not when uncoupled for all but the first 100 ms of the AP. Our findings demonstrate that gap junction coupling of ventricular myocytes to myofibroblasts may directly induce EADs and the probability of EAD induction correlates with the myofibroblast-myocyte gap junction coupling strength. Elucidating the mechanism of myofibroblast-induced arrhythmogenesis may suggest new therapeutic strategies for preventing ventricular arrhythmias based on inhibiting fibroblast proliferation and/or uncoupling fibroblasts from myocytes.

3060-Pos Board B165
Targeted Inhibition of Connexin 43 Hemichannels Blunts Ca^{2+}-Induced Intercellular Dyssynchrony and ATP Efflux in Hl-1 Cardiomyocyte Syncitia
Nicole C. Silvester, Hala Jundi, Archana Jayanthi, Steven R. Barberini-Jammaers, W Howard Evans, Christopher H. George.
Gap junctions (GJ) are essential conduits that underpin cell-to-cell coupling between cardiomyocytes and are formed from the coalescence of connexin (Cx) hemichannels from two close opposed cells. However, there is evidence supporting a (patho)physiologic role for 'unpaired' Cx hemichannels as they traffic through the plasma membrane (PM) to the GJ. We used beating HL-1 cardiomyocyte monolayers as a model system for GJ intercellular communication (GJIC) and to investigate the functional role of Cx hemichannels in syncitial behaviour. GJIC was quantified as an index of intercellular Ca^{2+} release synchrony and was reconciled with detailed spatio-temporal analysis of intracellular Ca^{2+} signalling. Ouabain-evoked Ca^{2+} perturbation inhibited GJIC in a dose-dependent manner and was associated with reduced cell viability. We hypothesised that the intercellular dyssynchrony and cell death linked to ouabain-induced Ca^{2+} dysfunction was exacerbated by aberrant Cx hemichannel opening that may also compromise cellular metabolism. Consistent with this concept, the magnitude of intracellular Ca^{2+} flux dysfunction correlated with ATP release from cells. Trans-PM ATP leak was attenuated by Gap20, a peptide corresponding to an intracellular loop motif in Cx43. Moreover, Gap20 reduced intracellular Ca^{2+} perturbation, improved intercellular synchrony and reduced cell death in ouabain-treated syncitia. The lack of efficacy of a peptide corresponding to a similar epitope in Cx26, and also following the conjugation of Gap20 to a high molecular weight dextran confirmed i) the specificity of this approach and ii) that peptide bioactivity is dependent on its entry into cells and interaction with the intracellular face of Cx. Our data provides evidence that altered cellular Ca^{2+} homeostasis opens Cx hemichannels and that this may accelerate the metabolic deterioration of cardiomyocytes and exacerbate cardiac dysfunction in situ.

3061-Pos Board B166
Binding Kinetics of Inter-Connexon Interaction
Felix Rico, Atsunori Oshima, Yoshinori Fujiyoshi, Peter Hinterdorfer, Simon Scheuring.
Gap junctions are pairs of hexameric half-channels called connexons, which coaxially dock to connect two adjacent cells, mediating both adhesion and channeling between cells in many types of tissue. Connexons are formed of six connexin proteins (Cx). Gap junctions form by interdigitating the two extracellular loops of each connexin. While gap junction structure and function has been widely characterized using different techniques, the binding affinity of the inter-connexon interaction remains unknown. The goal of this work was to determine the binding affinity of gap junctions using dynamic force spectroscopy atomic force microscopy (AFM). Among the residues that mediate interconnexon interaction, an exposed stretch of conserved amino acids 'NTVD' within the extracellular loop 2 (E2) has been identified. For dynamic force spectroscopy, we covalently linked mimetic peptides 'NTVD' that mimic loop E2 of Cx26 to the AFM tip, while Cx26 two-dimensional (2D) crystals were immobilized on a mica substrate. We report the first characterization of the binding strength of the gap junction interaction. Force curves at various retraction speeds were acquired to determine the dissociation kinetics of the peptide-Cx26 interaction, while adhesion probability measurements at different contact times revealed the binding kinetics. The relatively fast intrinsic dissociation rate (k_{off}) inferred a rather dynamic inter-connexon interaction, while the slow association rate (k_{on}) probably reflects the restricted mobility and degrees of freedom of the connexons in the densely packed organization observed in native gap junction plaques and the reduced flexibility and dimensions of the extracellular loops. Our results suggest that gap junction formation may occur before plaque formation.

3062-Pos Board B167
A Stochastic Model of Voltage-Gating of Connexin-Based Gap Junction Channels Containing Fast and Slow Gates
Nerijus Paulauskas, Henrikas Pranevicius, **Feliksas Bukauskas**.
Connexins (Cxs), a family of membrane proteins, form gap junction (GJ) channels that provide a direct pathway for electrical and metabolic cell-cell interaction. Each hemichannel in the GJ channel contains fast and slow gates sensitive to transjunctional voltage (V_j). The fast gate operates between open and residual states with conductances of $\gamma_{F,o}$ and $\gamma_{F,res}$, while the slow gate operates between open ($\gamma_{S,o}$) and fully closed ($\gamma_{S,closed}$=0) states; all γs rectify but at different degree. We developed a stochastic 16-state model (S16SM), which extends earlier reported S4SM (*Paulauskas et al., 2009*) and accounts operation of four gates in series, instead of two, to describe the gating properties of homotypic and heterotypic GJ channels. Operation of each gate depends on the state of three other gates in series do to their effect on the fraction of V_j that falls across the gate (V_G) and is determined by equilibrium constants, $K_i=e^{Ai\cdot(\Pi i\cdot VG-V0)}$, where A_i characterizes the sensitivity to voltage, V_0 is the voltage for K_i=1 and Π_i is gating polarity. S16SM allows to simulate kinetics of junctional current and junctional conductance (g_j) dependence on V_j for several frequently used experimental protocols: 1) consecutive V_j steps rising in amplitude, 2) slowly rising V_j ramps, and 3) series of V_j steps of high frequency. In addition, we have developed *universal* V_j protocol simulating freely selected forms of V_j consisting of an unlimited number of consecutively combined pulses and ramps of variable durations and amplitudes. The model was used to evaluate parameters of fast and slow gates of homo- and heterotypic GJs for experimentally measured g_j-V_j dependencies under normal/control and pathological conditions. The proposed S16SM was also used to evaluate gating properties of unapposed hemichannels residing in the non-junctional plasma membrane.

Voltage-gated K Channels - Permeation

3063-Pos Board B168
Ion Conduction in a Shaker Potassium Channel Mutant Having an Unusually High Single Channel Conductance
David Naranjo, Ignacio Valencia, Cristian Moscoso, Katherine Stack, Valeria Márquez, Ariela Vergara, Marcos Sotomayor, Fernando González-Nilo.
K-channels are endowed of a highly K^+ selective pore but are very diverse in single channel conductance, ranging from 3 to 300 pS. Because the selectivity filter is so conserved among K-channels, the origin of this diversity must be searched for in other parts of the pore. We studied the single-channel behavior of the Shaker K-channel with the P475D point mutation located at its internal entrance. In 100 mM K^+ solution, this variant has a unitary conductance 8-10 fold larger than wild type (Sukhareva et al. 2003. J. Gen. Physiol. 122:541,). We did single channel recording of the variants expressed in Xenopus oocytes and molecular dynamics simulations of channels modeled by homology with the Kv1.2-Kv2.1 chimera structure (Long et al. 2007. Nature. 450:376). Single channel conductance was measured in the interval −100/+150 mV between 50-1000 mM KMES. Below 300 mM KMES, single channel currents exhibit significant inward rectification, but at 1000 mM the I-V relation is nearly symmetrical. This channel is blocked by Mg^{2+} ions in a voltage independent fashion ($z\delta$ ~zero) at 50 mM KMES, but at 1000 mM KMES, $z\delta$ approaches ~0.5. These results suggest that internal K+ ions lock in Mg^{2+} inside the pore. To test, indirectly, the physical dimensions of the channel internal entrance, we

recorded voltage ramps in the interval ±200 mV in the presence of 2 M internal sucrose. In these conditions, outward currents become voltage independent at voltages >150 mV, providing size estimates consistent with the size of the internal mouth derived from the crystallographic structure. The ion density profile obtained from molecular dynamics simulations using an applied voltage reveals a high density of K+ ions near P475D.
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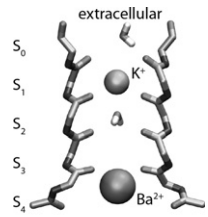
3064-Pos Board B169
Calculating Conductance and Size of the Entrance to the Inner Cavity of BK Channels with Side-Chain Replacement and a Two-Resistor Model
Yanyan Geng, Karl L. Magleby.
BK channels have the largest conductance (~250 pS) of all K+ selective channels. Previous studies suggest that residues E321/E324 in BK channels are located at the entrance to the inner cavity. We find that attachment of thiol reagents MPA and MTSET to E321C/E324C altered outward single-channel currents, suggesting that 321/324 face the ion conduction pathway. Therefore, substituting E321/E324 with different sized amino acids should change the size of the entrance to the inner cavity. We find that decreasing the size of the entrance decreases the conductance, whereas increasing the size of the entrance has little effect. Increasing [K+]i from 0.15 to 2.5 M negates differences in single-channel current associated with different side-chain volume. Plots of conductance vs. side-chain volume are approximated with a simple two-resistor model, where the ion conduction pathway is described by two resistors in series. R2 is a variable resistor, with resistance inversely proportional to the volume of the entrance to the inner cavity. R1 is a fixed resistor arising from the other parts of the conduction pathway including the selectivity filter. Fitting the data indicates that R1+R2 is ~5.4 gigaohm for glycine substitution, with an R1/R2 ratio of ~17, and effective radius and length of the entrance to the inner cavity of ~9.0 and 5.4 Å, respectively. (The volume of K+ and water are not taken into account.) The calculated size of the entrance to the inner cavity of BK channels is consistent with the crystal structure of large conductance bacterial MthK channels. These observations suggest that a large entrance to the inner cavity is required for the large conductance of BK channels, as decreasing the entrance size decreases the outward single-channel currents. Support: NIH-AR32805.

3065-Pos Board B170
Mechanism for Selectivity-Inactivation Coupling in KcsA Potassium Channels
Jason G. McCoy, Wayland Cheng, Ameer N. Thompson,
Crina M. Nimigean, Colin G. Nichols.
Potassium channels containing the GYG motif often diverge in their selectivity for monovalent cations, but the molecular basis is unknown. Using the prokaryotic potassium channel KcsA as our model, we have investigated the role of the interaction between glutamate 71 and aspartate 80, located behind the selectivity filter, in determining the selectivity of the channel as well as its influence on the conformation of the filter. In E71A KcsA channels, Na+ permeates at higher rates in both the presence and absence of K+, as seen with 86Rb+ and 22Na+ flux measurements. Single channel recordings indicate that Na+ ''punches through'' E71A KcsA channels at lower voltages than wild-type KcsA, and in the punchthrough regime the Na+-blocked current appears significantly larger. A crystal structure of E71A KcsA reveals that in contrast to what was seen for wild type KcsA, the selectivity filter does not collapse in the absence of K+, but instead assumes a ''flipped'' conformation. This flipped conformation is the same one observed in previous E71A KcsA structures in the presence of K+. The data reveal the importance of this E71-D80 interaction in both favoring inactivation and maintaining high K+ selectivity. We propose a molecular mechanism by which inactivation and K+ selectivity are linked, a mechanism that may also be at work in other channels containing the canonical GYG signature sequence.

3066-Pos Board B171
Structural Characterization of the Voltage Sensor Domain of the KvAP Channel Vectorially-Oriented within a Phospholipid Bilayer Membrane
Sanju Gupta, J. Liu, A. Tronin, J. Strzalka, J. Krepkiy, K. Swartz,
J. Kent Blasie.
Voltage-gated cation (Na^+, K^+) channels are responsible for the generation and propagation of action potentials in neurological signal transmission. Kv-channels are transmembrane proteins consisting of a homo-tetramer of 4 subunits that assemble about a 4-fold axis normal to the membrane plane to form the K^+ ion-selective pore. Each of the four subunits is comprised of six transmembrane helices, the S1-S4 helices forming the voltage-sensor domain (VSD) and the S5-S6 helices contributing to form the pore domain (PD). Despite several advances in the field, a complete understanding of the mechanism of electromechanical coupling interconverting the closed-to-open states is yet to be achieved. Positively charged arginine residues predominately in the S4 helix of the VSD are responsible for voltage sensing and the VSD's are arranged around the periphery of the PD in extensive contact with the lipid bilayer. This prompted us to focus initially on the structure of VSD itself within a phospholipid bilayer environment for the present study. A hydrated, phospholipid bilayer membrane environment has been reconstituted for the VSD of KvAP, vectorially oriented on the surface of inorganic multilayer substrates. This has been established by X-ray and neutron reflectivity (enhanced by interferometry), the latter employing a specifically deuterated phospholipid and water contrast variation, for the reconstituted membrane at both the solid-vapor and solid-liquid interfaces. This accomplishment now allows an investigation of the profile structure of the VSD within the lipid bilayer as a function of the applied transmembrane electric potential via x-ray reflectivity with millisecond time-resolution, employing high energy x-rays (> 20KeV) & pixel array detectors, and neutron reflectivity, employing selectively deuterium-labeled VSD proteins achieved via semi-synthesis. The same approach can be extended to the intact KvAP channel.

3067-Pos Board B172
KcsA Ion Affinity at an External Site Probed by Barium Block
Kene N. Piasta, Christopher Miller.
Block by Ba^{2+} is a distinctive property of K^+ channels since Ba^{2+}, a doubly charged analog for K^+, is electrostatically stabilized in the permeation pathway. Ba^{2+} block was used in BK channels as a tool to determine the equilibrium binding affinity for various ions at specific sites in the selectivity filter. In this work, we applied this approach to discrete block of single E71A KcsA channels, a non-inactivating mutant, in order to determine a thermodynamic measure of selectivity in a channel with abundant high-resolution structural information. We find at high concentrations of external K^+ the block time distribution is described by two distinct populations of Ba^{2+} block events. This argues there are at least two Ba^{2+} sites in the selectivity filter, fitting well with the published Ba^{2+} containing structure of KcsA where a Ba^{2+} ion resides approximately in S2 and S4. Utilizing a kinetic analysis of the blocking events as a function of external K^+, we determined the equilibrium dissociation constant of K^+ and other monovalent cations in an extracellular site, presumably S1, to arrive at a selectivity sequence for this particular site: Rb^+ (1 M) > K^+ (19 M) >> Na^+ (>1M). This represents an unusually high selectivity for K^+ over Na^+ with a $\Delta\Delta G^0$ of at least -7 kcal mol^{-1}. We are currently determining affinities for Li^+, NH_4^+, and Cs^+ at this site. The results fit well with other kinetic measurements of selectivity as well as with the many structures in various ionic conditions.

3068-Pos Board B173
QM/MM Modeling of Ba^{2+} Blockades in Potassium ion Channels
Christopher N. Rowley, Bogdan Lev, Sergei Noskov, Benoit Roux.
The robust selectivity of potassium channels for K^+ over Na^+ ions is a major component of the regulation of intracellular K^+ concentrations. The selectivity for K^+ was quantified through experiments measuring the Na^+ and K^+ dependence on Ba^{2+}-blockades,(1) indicating that K^+ has greater permeability by at least 150 fold. In thermodynamic terms, the relative binding free energy of Na^+ to the pore must be at least 3 kcal/mol less favorable than K^+. Na^+ vs K^+ free energy perturbation (FEP) simulations are consistent with this, although no simulations to date have modeled the actual Ba^{2+} blockade experiment. We have used MD simulations to calculate the relative binding energies of Na^+ and K^+ in the KcsA ion channel when the S4 site is occupied by Ba^{2+}. As Ba^{2+} is a strongly polarizing ion, we have used QM/MM FEP calculations using CHARMM interfaced to the deMon DFT code (2), as well as the polarizable Drude force field to correctly model the Ba^{2+}-filter interactions, with the aim of better interpreting the original selectivity experiments.



Schematic of ion binding in the selectivity filter.

1. J. Neyton, C. Miller, *J. Gen. Physiol.* 92: 549-567.
2. B. Lev et al., *J. Comp. Chem.*, 31: 1015-1023.

3069-Pos Board B174
Human *ETHER-A-Go-Go*-Related Gene (HERG) K^+ Channel Inhibition by the Antidepressant Paroxetine
Hee-Kyung Hong, **Su-Hyun Jo**.
Paroxetine is a selective serotonin reuptake inhibitor (SSRI) for psychiatric disorders that can induce QT prolongation, which may lead to *torsades de*